POTENTIAL BIOLOGICAL CONTROL OF *LANTANA CAMARA* IN THE GALAPAGOS USING THE RUST *PUCCINIA LANTANAE*

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ASBTRACT

Laboratory experiments were carried out in England to test the specificity and environmental requirements of a Peruvian isolate of the fungus *Puccinia lantanae* Farlow, known to attack the invasive plant *Lantana camara* L. a serious problem in Galapagos. Eight species of plants representing five families were inoculated with the fungus and kept in a dew chamber for 48 hours. *Lantana peduncularis* Andersson and *Lantana camara* were sourced from Galapagos; other species related to *Lantana* were sourced from other places. Dew periods of 5, 8, 11, 14, and 20 hours were tested to determine the period necessary for basidiospore formation and host infection. Only *Lantana camara* from Galapagos and Peru developed visible symptoms 6 days after inoculation and after 15 days sori were fully developed. No non-target species developed macroscopic symptoms. Most importantly the rust did not attack the closest host relative from Galapagos, the endemic *Lantana peduncularis*. Eight hours in the dew chamber was enough to induce basidiospore formation and host infection, but times up to 20 hours induced progressively more sori. Although we have not completed yet the experiments to determine the host range specificity, *P. lantanae* shows promise as a biocontrol agent for *Lantana camara* in Galapagos.

KEY WORDS: *Lantana peduncularis*, *Lantana camara*, *Puccinia lantanae*, rust, biocontrol, Galapagos

RESUMEN

Se realizaron experimentos de laboratorio en Inglaterra para determinar el nivel de especificidad y requerimientos ambientales del hongo *Puccinia lantanae* Farlow como potencial agente de control biológico de la especie invasora *Lantana camara* L. en Galápagos. Ocho especies de plantas pertenecientes a cinco familias relacionadas a *L. camara*, fueron inoculadas con pústulas del hongo y mantenidas durante 48 horas en la cámara de roció. *Lantana peduncularis* Andersson y *Lantana camara* fueron colectadas en Galápagos, las otras especies relacionadas con Lantana se obtuvieron en otros lugares. Para determinar el periodo de roció necesario para la producción de basidioesporas, las plantas de *L. camara* fueron inoculadas y sometidas a diferentes periodos de rocio (5, 8, 11, 14, 20 horas). *Puccinia lantanae* se desarrollo y afectó únicamente a *L. camara* proveniente de Galapagos y Perú. Los síntomas aparecieron seis días después de la inoculación y a los 15 días las pústulas estuvieron completamente desarrolladas. No se detectaron síntomas macroscopicos en las otras especies, principalmente en la endémica *L. peduncularis* que es la especie mas cercana. *Puccinia lantanae* fue capaz de esporular e infectar *L. camara* luego de ocho horas de roció; el mayor grado de infección y desarrollo de pústulas se obtuvo dentro de las 20 horas de roció. No se han realizado todas las pruebas necesarias para determinar si el nivel de especificidad es adecuado para liberar el agente, sin embargo hasta el momento, *P. lantanae* se muestra como un potencial agente de control biológico de *L. camara* para Galápagos.

INTRODUCTION

Lantana camara (Verbenaceae), an ornamental shrub, native to tropical America, is now found in most tropical and subtropical regions of the world. It is not only widespread but it is also generally considered to be a major pest of agricultural and natural areas (Thaman 1974). The *L. camara* complex will tolerate a wide range of climates. It can be found at altitudes between sea level and up to 2000 meters, and between 45° N to 45° S. It flourishes in both dry and wet regions, growing on mountain slopes, along coastal areas and in valleys. It is somewhat shade tolerant and thus can become the dominant understory plant in open forest and tropical tree crops in its weedy range (Holm et al. 1977). Reproduction is by seeds that are spread via frugivorous birds, and short distance spread is by the rooting of horizontal stems in contact with the soil.

Lantana camara was first introduced as an ornamental into Floreana Island in the Galapagos Archipelago in 1938 (Cruz et al. 1986), and has since spread or been carried to other islands, including Santa Cruz. The dense thickets created by this invasive weed impact not only the indigenous flora but also fauna (Hamann 1984; Cruz et al. 1986). There is evidence, for example, that it is affecting the nesting habitats of the endangered dark-rumped petrel, *Pterodroma phacopygia* (Cruz et al. 1986).

Lantana camara was the first weed ever targeted for classical biological control at the turn of the century. The first attempt at the biological control of lantana began in 1902, when 23 insect species were imported into Hawaii from Mexico. Eight of these species were established (Perkins & Swezey 1924). A total of 36 insect species has since been released in 33 countries (Julien & Griffiths 1998), but control in Hawaii, as well as in other parts of the world, has only been partially successful (Taylor 1989). This has mainly been due to the genetic diversity, and hence environmental adaptability, of the weedy biotypes which outstrip those of its natural enemies. New biocontrol agents are still being evaluated and released, including pathogens. A broad range of pathogens has been recorded infecting *L. camara* in its native range (Barreto et al. 1995). Three fungal agents have been released so far: a species of *Septoria* was released in Hawaii, originally from Ecuador (Trujillo 1995), a rust *Prospodium tuberculatum* (ex Brazil) was released in Australia in 2001, and a leaf spot pathogen, *Mycovellosiella lantanae* var. *lantanae* (ex Florida, USA) was released in South Africa, also in 2001. The impact of these agents is still pending. *Puccinia tuberculatum* was screened against the invasive and native species of Lantana from the Galapagos at CABI Bioscience, but it was found to infect, albeit mildly, the native lantana, and was discounted as a potential agent. A number of other candidate pathogens have been identified with excellent potential, including a stem and leaf rust, *Puccinia lantanae* (Barreto et al. 1995).

Puccinia lantanae Farlow (Basidiomycotina, Uredinales) occurs in tropical

and subtropical regions of America: From Mexico and Florida, through the Caribbean and as far South as Argentina. *Puccinia lantanae* has been recorded from a number of *Lantana* spp., but there is evidence of distinct races that are only capable of attacking single species, and are even specific to biotypes within that species. This rust is recorded as a microcyclic (only teliospores and basidiospores in the life cycle) and autoecious (completes life cycle on one host species) species. The teliospores remain in the sorus on the host plant, and are not released. Under conditions of high humidity, teliospores germinate and produce basidiospores that are released from the teliospores. These infect fresh plant material, from which more teliospores result, and hence complete the life cycle.

METHODS

Plant material and fungal inoculations

Plants species used in the experiment were grown from stem cuttings from the CABI Bioscience stock plant collection. *Lantana camara* and *Lantana peduncularis* were collected originally from the Galapagos Islands (Santa Cruz). Using rooting powder, stem cuttings were planted in pots containing substrate (John Innes no. 2). Plants were kept in a quarantine glasshouse set at a minimum temperature of 20 °C with 12 hours of artificial light and watered everyday.

The rust *Puccinia lantanae* used was taken from the CABI Bioscience specimen collection held on living plants (isolate reference number W1914). The fungus, like all rusts is a biotroph and therefore culturing can only be done *in vivo*.

To inoculate the experimental plants for host range testing and assessment of the minimum dew period requirement, sori of *Puccinia lantanae* were suspended over new shoots; between two to four shoots were targeted for each potted plant. The small piece of plant tissue containing the sori of teliospores, was attached to small Petri dish using petroleum jelly (Vaseline). Care was taken that no Vaseline was deposited on the fungal material. Petri dishes were attached to a small stick a distance of 2 cm above the young leaves, making sure that the teliospores were directly above the leaf, so the basidiospores are released onto the potentially susceptible part of the plant. (Koutsidou 2000). The target area was usually the four youngest leaves of any given stem. The inoculated stems were marked by tying a string to the stem. Target plants were watered and the leaves were wetted with a fine mist of sterile distilled water before putting them in the artificial dew chamber (Mercia Scientific, Birmingham, UK). All *in vivo* experiments were done in a quarantine greenhouse at CABI Bioscience, Ascot. United Kingdom.

There were two experiments described below:

Symptom Development and Host Range Specificity

For the host range specificity experiments, eight species from five families were used (Table 1). All test plants species were inoculated with the rust *Puccinia*

TABLE 1. Host range test species list and results of host specificity testing of *Puccinia lantanae*.

Family	Species	Provenance	Native in	Susceptibility rating
Bignonaceae	*Tecomanthe hilli*	Australia	Australia	0
Boraginaceae	*Cordia dichotoma*	Australia	Australasia	0
Lamiaceae	*Plectranthus parviflorus*	Australia	Australasia	0
Lamiaceae	*Vitex triflora*	Australia	South America	0
Lamiaceae	*Gmelina leichhardtii*	Australia	Australia	0
Verbenaceae	*Lantana camara*	Galapagos	South America	3
Verbenaceae	*Lantana camara*	Peru	South America	3
Verbenaceae	*Lantana montevidensis*	Australia	South America	0
Verbenaceae	*Lantana pedunculoris*	Galapagos	Galapagos	0

lantanae using the method described above. At least three replicate plants were inoculated per species. Plants were incubated at 20° C for 48 hours in a dew simulation chamber to induce teliospore germination, basidiospores formation, and provide an optimum environment for potential plant infection.

Symptoms were recorded according to a rating system devised to assess the susceptibility of plant species to *P. lantanae* based on the visible symptoms (Koutsidou 2000): **0** No macroscopic symptoms; **1** Chlorosis on the leaf surface; **2** Restricted sporulation (sorus diameter <2mm), and; **3** Abundant sporulation (Sorus diameter >2mm).

Dew Period Requirements

Lantana camara plants (from Galapagos) were inoculated with the rust *Puccinia lantanae* using the method described above, although one sorus per shoot was used as the standard inoculum, of 5 mm diameter. Three or more shoots were inoculated per replicate plant and a mean number of sori per shoot taken for each plant. Dew period treatments of 5, 8, 11, 14, and 20 hours were compared, with two replicate plants per treatment. The dew chamber was set at 20° C. After this treatment, the inoculum was removed and plants moved into the quarantine glasshouse and pots watered normally avoiding the wetting of leaves. Plants were checked regularly for macrosymptoms and sorus development.

RESULTS

Symptom Development and Host Range Specificity

The first appearance of symptoms of *P. lantanae* infection on the *L. camara* from Galapagos, occurred 6-7 days after the inoculation, as small chlorotic spots. These spots enlarged, and after approximately 13-15 days the first symptom of sporulation i.e. sori became apparent. The size of the sori on leaves differed from between 1 mm to 6 mm in diameter. In general, the younger the leaves (approximately <5mm diameter) were at inoculation the larger the sori that formed. Leaves that had already partially expanded before infection produced the

smaller sori. However, high densities of sori on a leaf also resulted in smaller average sorus size. No sporulation was observed on leaves that were fully expanded at inoculation. Sporulation occurred mainly on the lower surface of the leaf. When the density of sori was high, very premature leaf abscission was observed (around 13 days after inoculation). If the density of sori was lower, a necrotic area formed around them, which increased until earlier than normal leaf fall, but after full rust symptom expression. Infection often occurred on stems and petioles also.

Table 1 gives the results of the host specificity testing. Although this is a limited host range test, the results suggest that *P. lantanae* is host specific to *L. camara*. *Puccinia lantanae* was not able to infect nor sporulate on any of the other seven related species used in the experiments. Even the most closely related species *L. peduncularis* (Galapagos) and *L. montevidensis* were resistant to *P. lantanae*, suggesting strong host-specificity.

Dew Period Requirements

Puccinia lantanae was able to sporulate and infect *L. camara* plants after only 8 hours of dew. Maximum infection and sori development was obtained at or after 20 hours of dew (see Fig. 1).

Figure 2 shows the different levels of infection by *P. lantanae* after different lengths of dew period. Clearly, more basidiospores are released over a longer period of time in humid conditions.

DISCUSSION

Puccinia lantanae isolate W1914 from Peru seems to be significantly more destructive to *Lantana camara* than other pathotypes of *P. lantanae*, that are frequently observed throughout the native range of the plant. Previous records of isolates of *P. lantanae* report that the pathogen only infects leaves (Barreto et al. 1995). The fact that the isolate W1914 can also infect petioles and stem means that the rust is much more damaging to the weed and is therefore a better potential biological control agent than originally estimated. Whole branches may drop as a result of stem infection and infection of the leaves can be very severe. Disease symptoms start to appear 5-7 days after inoculation and sori can grow up to 6 mm in diameter suggesting a rapid and destructive infection of this rust. This rust is able to release the basidiospores that can infect fresh host tissue within the first 8 hours of a dew period however longer periods of humidity favor it.

Puccinia lantanae seems to be a promising biological control agent to target *L. camara* from the Galapagos Islands. Nevertheless it is necessary to continue with the host range specificity test using the related native and endemic species from Galapagos to avoid doubts about non-target species effects.

In addition, more collections of *L. camara* from Galapagos need to be made

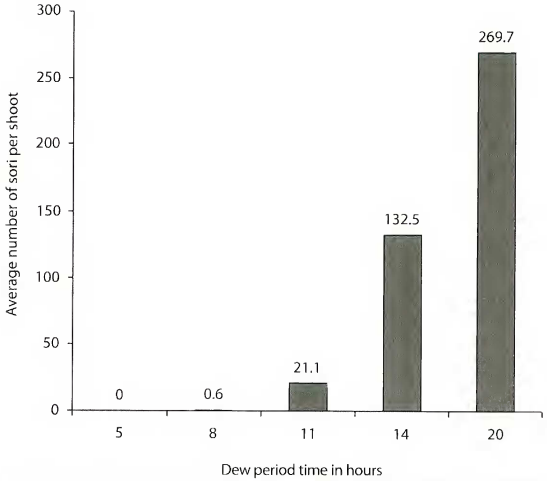

FIG. 1. Average number of sori per shoot developed within the five dew period time treatments.

to ensure that the rust is able to attack all the possible forms of the weed that may occur in Galapagos. There is evidence (from observations on flower color and plant growth form), that this weed has a narrow genetic base on the islands, and hence this rust is likely to infect all populations. Although only limited host specificity testing has been undertaken, the results suggest that this isolate is specific to *L. camara*.

Classical releases of rusts with the same type of life cycle as *P. lantanae* (e.g. microcyclic), show that such short-cycled rusts spread swiftly through and between plant populations (Morin et al. 1996.). The impact of successful classical biological control agents on woody weed species, such as *L. camara*, has tended to take a decade or more to be demonstrated, and often a suite of natural enemies is required. It is anticipated that due to the damaging nature of the rust and the short generation time, the impact may be observed sooner than is normal, and that this single agent may provide effective control.

In the subsequent glasshouse based tests of this agent, untreated (not inoculated) plants of *L. camara* should be compared with treated individuals, in

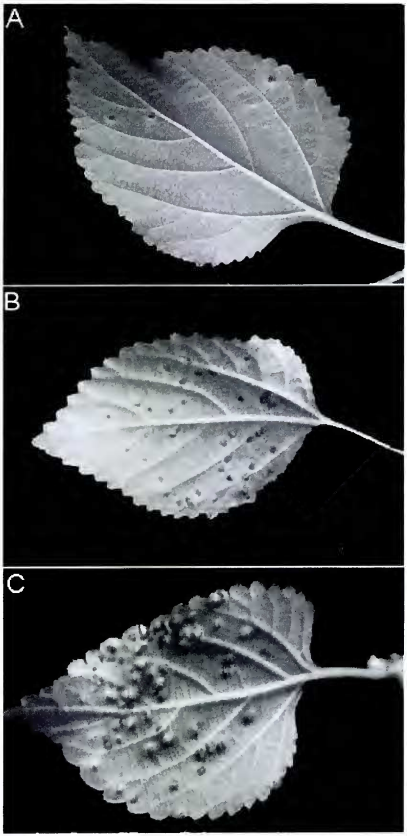

FIG. 2. Levels of infection of *Puccinia lantanae* as result of the different dew period treatments. A. eight hours, B. fourteen hours, C. twenty hours in the dew chamber.

order to investigate the effect of the rust on plant growth and survival. This may help give an indication of the possible level of impact of the agent in the field, although this is known to be quite difficult to study with woody species in controlled conditions.

Lantana camara is difficult to control and appears impossible to eradicate due to the wide range it occupies in the Galapagos. Biological control is a realistic management option. This weed has been studied for over a century as a classical biological control target. Although success has been limited, the rust *Puccinia lantanae* constitutes a new method and a potentially effective agent to try in Galapagos Islands.

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